

FIGURE 1

cDNA sequence of wild type amFP486

ATGGCTCTTCAAACAAGTTATCGGAGATGACATGAAAATGACCTACCATATGGATG  
GCTGTGTCATGGCATTACTTACCGTCAAAGGTGAAGGCAACGGGAAGCCATACGA  
AGGGACGCAGACCTCGACTTTAAAGTCACCAGGCCAACGGTGGGCCCTGCATT  
TCCTTGACATACTATCTACAGTGTCAAGTATGAAATCGATGCTTACTGCGTATC  
CTACCACTATGCCGACTATTCAAACAAGCATTCTGACGGAATGTCATATGAAAG  
GACTTTACCTATGAAGATGGAGGAGTTGCTACAGCCAGTTGGAAATAAGCCTTAAA  
GGCAACTGCTTGAGCACAAATCCACGTTCATGGAGTGAACCTTCCTGCTGATGGAC  
CTGTGATGGCGAAGATGACAACCTGGTGGGACCCATCTTGAGAAAATGACTGTCTG  
CGATGGAATATTGAAGGGTGATGTCACCGCGTCTCATGCTGCAAGGAGGTGGCAAT  
TACAGATGCCATTCCACACTTCTTACAAGACAAAAAAACCGGTGACGATGCCACCAA  
ACCATGCGGTGGAACATCGCATTGCGAGGACCGACCTGACAAAGGTGGCAACAGTGT  
TCAGCTGACGGAGCACGCTGTTGCACATATAACCTCTGTTGTCCCTTC (SEQ ID  
NO:01)

amino acid sequence of wild type amFP486

MALSNKFIGD DMKMTYHMDG CVNHYFTVK GEGNGKPYEG TQTSTFKVTM ANGGPLAFSF  
DILSTVFKYG NRCFTAYPTS MPDYFKQAFP DGMSYERTFT YEDGGVATAS WEISLKGNCF  
EHKSTFHGVN FPADGPVMAK MTTGWDPSFE KMTVCDGILK GDVTAFLMLQ GGGNYRCQFH  
TSYKTKKPVT MPPNHAVEHR IARTDLDKGG NSVQLTEHAV AHITSVVPF  
(SEQ ID NO:02)

H  
O  
S  
D  
G  
S  
P  
A  
D  
R  
G  
S  
A  
R

Figure 2

### cDNA sequence of zFP506

ATGGCTCAGTCAAAGCACGGTCTAACAAAAGAAATGACAATGAAATACCGTATGGAAGGGTGC  
GTCGATGGACATAAATTGTGATCACGGGAGAGGCATTGGATATCGTTCAAAGGAAACAG  
GCTATTAATCTGTGTGGTCGAAGGTGGACCATTGCCATTGCCGAAGACATATTGTCAGCT  
GCCTTATGTACGGAAACAGGGTTTCACTGAATATCCTCAAGACATAGCTGACTATTCAG  
AACTCGTGCCTGCTGGTTATACATGGGACAGGTCTTCTTTGAGGATGGAGCAGTTGC  
ATATGTAATGCAGATATAACAGTGAGTGTGAAGAAAACATGCATGTATCATGAGTCCAAATT  
TATGGAGTGAATTTCCTGCTGATGGACCTGTGATGAAAAAGATGACAGATAACTGGGAGCCA  
TCCTGCGAGAAGATCATACCACTAACGTAAGCAGGGATATTGAAAGGGATGTCCTCATGTAC  
CTCCTTCTGAAGGATGGTGGCGTTACGGTGCCAATTGACACAGTTACAAAGCAAAGTCT  
GTGCCAAGAAAGATGCCGACTGGCACTTCATCCAGCATAAGCTCACCGTGAAGACCGCAGC  
GATGCTAAGAATCAGAAATGGCATCTGACAGAACATGCTATTGCACTCCGGATCTGCATTGCC  
(SEQ ID NO:03)

### amino acid sequence of zFP506

MAQSKHGLTK EMTMKYRMEG CVDGHKFVIT GEGIGYPFKG KQAINLCVVE GGPLPFAEDI LSAAFNYGNR VFTEYPQDIA  
DYFKNSCPAG YTWDRSFLFE DGAVCICNAD ITVSVEENCM YHESKFYGVN FPADGPVMKK MTDNWEPSCE KIIPVPKQGI  
LKGDVSMYLL LKDGGLRQC FDTVYKAKSV PRKMPDWHFI QHKLTREDRS DAKNQKWLTT EHAIASGSAL P  
(SEQ ID NO:04)

Figure 3

cDNA sequence of zFP538

gagttgagtt tctcgacttc agttgtatca attttgggc atcaagcgat ctatttcaa  
catggctcat tcaaagcacg gtctaaaaga agaaatgaca atgaaatacc acatggaaagg  
gtgcgtcaac ggacataaat ttgtgatcac gggcgaaggc attggatatac cgttcaaagg  
gaaacagact attaatctgt gtgtgatcga agggggacca ttgccatttt ccgaagacat  
attgtcagct ggctttaagt acggagacag gatttcact gaatatcctc aagacatagt  
agactattc aagaactcgt gtcctgtgg atatacatgg ggcaggtctt ttctctttga  
ggatggagca gtctgcataat gcaatgtaga tataacagtg agtgtcaaag aaaactgcata  
ttatcataag agcatattta atggaatgaa ttttcctgct gatggacctg tgatgaaaaa  
gatgacaact aactgggaag catcctgcga gaagatcatg ccagtaccta agcaggggat  
actgaaaggg gatgtctcca tgcgttctt tctgaaggat ggtggcggtt accgggtccca  
gttcgacaca gtttacaaag caaaagtctgt gccaagtaag atgcccggagt ggcacttcat  
ccagcataag ctcctccgtg aagaccgcag cgatgctaag aatcagaagt ggcagctgac  
agagcatgct attgcattcc cttctgcattt ggcctgataaa gaatgttagtt ccaacatttt  
aatgcatgtg cttgtcaattt attctgataaa aatgttagtt gagttgaaaa cagacaagta  
caaataaaagc acatgtaaat cgtct (SEQ ID NO:05)

amino acid sequence of zFP538

Met Ala His Ser Lys His Gly Leu Lys Glu Glu Met Thr Met Lys  
Tyr His Met Glu Gly Cys Val Asn Gly His Lys Phe Val Ile Thr  
Gly Glu Gly Ile Gly Tyr Pro Phe Lys Gly Lys Gln Thr Ile Asn  
Leu Cys Val Ile Glu Gly Gly Pro Leu Pro Phe Ser Glu Asp Ile  
Leu Ser Ala Gly Phe Lys Tyr Gly Asp Arg Ile Phe Thr Glu Tyr  
Pro Gln Asp Ile Val Asp Tyr Phe Lys Asn Ser Cys Pro Ala Gly  
Tyr Thr Trp Gly Ser Phe Leu Phe Glu Asp Gly Ala Val Cys Ile  
Cys, Asn Val Asp Ile Thr Val Ser Val Lys Glu Asn Cys Ile Tyr  
His Lys Ser Ile Phe Asn Gly Met Asn Phe Pro Ala Asp Gly Pro  
Val Met Lys Lys Met Thr Thr Asn Trp Glu Ala Ser Cys Glu Lys  
Ile Met Pro Val Pro Lys Gln Gly Ile Leu Lys Gly Asp Val Ser  
Met Tyr Leu Leu Leu Lys Asp Gly Gly Arg Tyr Arg Cys Gln Phe  
Asp Thr Val Tyr Lys Ala Lys Ser Val Pro Ser Lys Met Pro Glu  
Trp His Phe Ile Gln His Lys Leu Leu Arg Glu Asp Arg Ser Asp  
Ala Lys Asn Gln Lys Trp Gln Leu Thr Glu His Ala Ile Ala Phe  
Pro Ser Ala Leu Ala (SEQ ID NO:06)

## FIGURE 4

All mutants are derived from drFP583 (called "pink" or FP6.) by random mutagenesis

The mutants E57 and AG4 are derivative from E5

Mutant: E5 = V105A, S197T Phenotype: in *E.coli* seen as Green overnight, matures to Red over 24h at 37°C (final peaks ratio Red vs. Green is 75:25); folding is faster than FP6.

Mutant: E8 = N42H Phenotype: always has two peaks Green & Red in approx. 60:40; folding is faster than E5 (about 8h at 37°C)

Mutant: E83 = N42H, V71A, I180V Phenotype: always has two almost equal peaks Green & Red; folding is the same as for E8

Mutant: E5up = V105A Phenotype: seen as Red from the beginning; folding is faster than E5 (about 12-16h) Almost no Green peak at final point of maturation

Mutant: E57 = V105A, I161T, S197A Phenotype: at common is like E5up but folding is more faster (no more than 8-10h) Very small Green peak at final point of maturation (less than 5%)

Mutant: E5down = S197T Phenotype and folding rate are exactly the same as for E5

Mutant: AG4 = V71M, V105A, S197T Phenotype: Very bright Green, no Red at all (even at the beginning); folding is faster than E5 (no more than 16h)

Mutant: AG4 = V71M, V105A, Y120H, S197T Phenotype: at common is like AG4, but more brighter (approx. twice) one.

1	Met Arg Ser Ser Lys Asn Val Ile Lys Glu Phe Met Arg Phe Lys Val	16
1	ATG CGC TCC TCC AAG AAC GTC ATC AAG GAG TTC ATG CGC TTC AAG GTG	48
17	Arg Met Glu Gly Thr Val Asn Gly His Glu Phe Glu Ile Glu Gly Glu	32
49	CGC ATG GAG GGC ACC GTG AAC GGC CAC GAG TTC GAG ATC GAG GGC GAG	96
	His (CAC) for E8 and E83	
33	Gly Glu Gly Arg Pro Tyr Glu Gly His Asn Thr Val Lys Leu Lys Val	48
97	GGC GAG GGC CGC CCC TAC GAG GGC CAC <u>AAC</u> ACC GTG AAG CTG AAG CTG	144
49	Thr Lys Gly Gly Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln	64
145	ACC AAG GGC GGC CCC CTG CCC TTC GCC TGG GAC ATC CTG TCC CCC CAG	192
	Met (ATG) for AG4 and AG45/Ala (GCG) for E83	
65	Phe Gln Tyr Gly Ser Lys Val Tyr Val Lys His Pro Ala Asp Ile Pro	80
193	TTC CAG TAC GGC TCC AAG <u>GTG</u> TAC GTG AAG CAC CCC GCC GAC ATC CCC	240
81	Asp Tyr Lys Lys Leu Ser Phe Pro Glu Gly Phe Lys Trp Glu Arg Val	96
241	GAC TAC AAG AAG CTG TCC TTC CCC GAG GGC TTC AAG TGG GAG CGC GTG	288
	Ala (GCG) - for E5, E57, AG4 and AG45	
97	Met Asn Phe Glu Asp Gly Gly Val Val Thr Val Thr Gln Asp Ser Ser	112
289	ATG AAC TTC GAG GAC GGC GGC <u>GTG</u> ACC GTG ACC CAG GAC TCC TCC	336
	His (CAC) - for AG45	
113	Leu Gln Asp Gly Cys Phe Ile Tyr Lys Val Lys Phe Ile Gly Val Asn	128
337	CTG CAG GAC GGC TGC TTC ATC <u>TAC</u> AAG GTG AAG TTC ATC GGC GTG AAC	384
129	Phe Pro Ser Asp Gly Pro Val Met Gln Lys Lys Thr Met Gly Trp Glu	144
385	TTC CCC TCC GAC GGC CCC GTG ATG CAG AAG ACC ATG GGC TGG GAG	432
145	Ala Ser Thr Glu Arg Leu Tyr Pro Arg Asp Gly Val Leu Lys Gly Glu	160
433	GCC TCC ACC GAG CGC CTG TAC CCC CGC GAC GGC GTG CTG AAG GGC GAG	480
	Thr (ACC) for E57	
161	Ile His Lys Ala Leu Lys Leu Lys Asp Gly Gly His Tyr Leu Val Glu	176
481	<u>ATC</u> CAC AAG GCC CTG AAG CTG AAG GAC GGC CAC TAC CTG GTG GAG	528
	Val (GTC) for E83	
177	Phe Lys Ser Ile Tyr Met Ala Lys Lys Pro Val Gln Leu Pro Gly Tyr	192
529	TTC AAG TCC <u>ATC</u> TAC ATG GCC AAG AAG CCC GTG CAG CTG CCC GGC TAC	576
	Thr (ACC) for E5, AG4 and AG45/Ala (GCC) for E57	
193	Tyr Tyr Val Asp Ser Lys Leu Asp Ile Thr Ser His Asn Glu Asp Tyr	208
577	TAC TAC GTG GAC <u>TCC</u> AAG CTG GAC ATC ACC TCC CAC AAC GAG GAC TAC	624
209	Thr Ile Val Glu Gln Tyr Glu Arg Thr Glu Gly Arg His His Leu Phe Leu ***	229
625	ACC ATC GTG GAG CAG TAC GAG CGC ACC GAG GGC CGC CAC CAC CTG CTG TAA	678
	(SEQ ID NO:07 & 8)	

## FIGURE 5

## Amino Acid and Nucleotide Sequence for asFP600

ATGGCTCCTTTAAAGAAGACTATGCCCTTAAGACGACCATTGAAGGGACGGTTAATGGCCAC  
TACTTCAAGTGTACAGGAAAAGGAGAGGGCAACCCATTGAGGGTACGCAGGAAATGAAGATAGAG  
GTCATCGAAGGAGGTCCATTGCCATTGCCACATTGTCAACGAGTTGTATGTACGGTAGT  
AAGGCCTTCATCAAGTATGTGTCAAGGATTCTGACTACTTCAAGCAGTCTTCCCTGAAGGTTTT  
ACTTGGAAAGAACCAACCTACGAGGATGGAGGCTTCTTACAGCTCATCAGGACACAAGCTA  
GATGGAGATTGCCTCGTTACAAGGTCAAGATTCTGGTAATAATTTCCTGCTGATGGCCCCGTG  
ATGCAGAACAAAGCAGGAAGATGGGAGCCATCCACCGAGATAGTTATGAAGTTGACGGTGTCTG  
CGTGGACAGTCTTGATGGCCCTTAAGTGCCTGGTCGTCACTGACTTGCCTCTCCATACT  
ACTTACAGGTCCAAAAAACAGCTGCTGCCTGAAGATGCCAGGATTCATTTGAAGATCATCGC  
ATCGAGATAATGGAGGAAGTTGAGAAAGGCAAGTGCTATAAACAGTACGAAGCAGCAGTGGGCAGG  
TACTGTGATGCTGCTCCATCCAAGCTTGGACATAAC (SEQ ID NO:09)

### Amino acid

MASFLKKTMP FKTTIEGTVN GHYFKCTGKG EGNPFEGTQE MKIEVIEGGP LPFAFHILST  
SCMYGSKTFI KYVSGIPDYF KQSFPEGFTW ERTTTYEDGG FLTAHQDTSL DGDCLVYKVK  
ILGNNFPADG PVMQNKAAGRW EPATEIVYEV DGVLRGQSLM ALKCPGGRHL TCHLHTTYRS  
KKPAAALKMP GFHFEDHRIE IMEEVEKGKC YKQYEAAVGR YCDAAPSKLG HN (SEQ ID  
NO:10)

Figure 6

## Sequence of humanized 6/9 hybrid gene and 6/9-Q3 mutant

for 6/9-2G and 6/9-03 CAG (0)

(SEQ ID NO:11 & 12)

Figure 7

## Nucleic acid sequence FP6 (E57)-NA

ATGGCCCTCCTCCGAGAACGTCATCACCGAGTTCATGCGCTTAAGGTGCGCATGGAGGGCACCGTGA  
ACGGCCACGAGTCGAGATCGAGGGCGAGGGCGAGGGCCCGCCCTACGAGGGCCACAACACCGTG  
AAGCTGAAGGTGACCAAGGGCGCCCCCTGCCCTCGCCTGGACATCCTGTCCCCCAGTTCCAGT  
ACGGCTCCAAGGTGTACGTGAAGCACCCCCGCCGACATCCCCGACTACAAGAACGCTGTCCCTCCCCGA  
GGGCTTCAGTGGAGCGCGTGTAGAACCTCGAGGGACGGCGCGTGGCGACCGTGACCCAGGACTC  
CTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGAAGTTCATGGCGTGAACTTCCCCCTCCGACGGC  
CCCGTGATGCAGAAGAACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCCGGACGGC  
GTGCTGAAGGGCGAGACCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGAGTT  
AAGTCCATCTACATGGCCAAGAACGCCCCGTGCAGCTGCCCGGCTACTACTACGTGGACGCCAAGCTGG  
ACATCACCTCCCACAACGAGGAGCTACACCATCGTGGAGCAGTACGAGCGCACCGAGGGCCGCCACCA  
CCTGTTCCCTG (SEQ ID NO:13)

Figure 8

### DNA sequence (ORF) of E5-NA

ATGGCCCTCTCCGAGAACGTATCACCGAGTTATGCGCTTCAAGGTGCGATGGAGGGCACCGTGA  
ACGGCCACGAGTTGAGATCGAGGGCGAGGGCGAGGGCCGCCCCCTACGAGGGCCACAACACCGTGA  
AAGTTGAAGGTGACCAAGGGCGGGCCCTGCCCCCTCGCCTGGACATCCTGTCCCCCAGTTCCAGT  
ACGGCTCCAAGGTGTACGTGAAGCACCCCGCCGACATCCCCGACTACAAGAACGCTGTCCCCCGA  
GGGCTTCAAGTGGGAGCGCGTGTGAACCTCGAGGACGGCGCGTGGCGACCGTGACCCAGGACTC  
CTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGAAGTTCATCGGCGTGAACCTCCCCCTCCGACGGC  
CCCGTGATGCAGAAGAACCATGGGCTGGGAGGCCTCCACCGAGGCCCTGTACCCCCCGCAGCGC  
GTGCTGAAGGGCGAGATCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTT  
AAGTCCATCTACATGGCCAAGAACGCCGTGCAGCTGCCGGCTACTACTACGTGGACACCAAGCTGG  
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAGCAGTACGAGCGCACCGAGGGCGCCACCA  
CCTGTTCTGTAA (SEQ ID NO:14)

**Figure 9**

Non-aggregating mutant FP3-NA was generated from zFP506-N65M (non-humanized version). In comparison with zFP506-N65M, FP3-NA contains two additional amino acid substitutions - K5E and K10E. Also, one accidental nucleotide substitution was introduced due to PCR mistake (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

GGA TCC GCT CAG TCA GAG CAC GGT CTA ACA GAA GAA ATG ACA ATG AAA  
BamHI A Q S E H G L T E E M T M K

TAC CGT ATG GAA GGG TGC GTC GAT GGA CAT AAA TTT GTG ATC ACG GGA  
Y R M E G C V D G H K F V I T G

GAG GGC ATT GGA TAT CCG TTC AAA GGG AAA CAG GCT ATT AAT CTG TGT  
E G I G Y P F K G K Q A I N L C

GTG GTC GAA GGT GGA CCA TTG CCA TTT GCC GAA GAC ATA TTG TCA GCT  
V V E G G P L P F A E D I L S A

GCC TTT ATG TAC GGA AAC AGG GTT TTC ACT GAA TAT CCT CAA GAC ATA  
A F M Y G N R V F T E Y P Q D I

GTT GAC TAT TTC AAG AAC TCG TGT CCT GCT GGA TAT ACA TGG GAC AGG  
V D Y F K N S C P A G Y T W D R

TCT TTT CTC TTT GAG GAT GGA GCA GTT TGC ATA TGT AAT GCA GAT ATA  
S F L F E D G A V C I C N A D I

ACA GTG AGT GTT GAA GAA AAC TGC ATG TAT CAT GAG TCC AAA TTC TAT  
T V S V E E N C M Y H E S K F Y

GGA GTG AAT TTT CCT GCT GAT GGA CCT GTG ATG AAA AAG ATG ACA GAT  
G V N F P A D G P V M K K M T D

AAC TGG GAG CCA TCC TGC GAG AAG ATC ATA CCA GTA CCT AAG CAG GGG  
N W E P S C E K I I P V P K Q G

ATA TTG AAA GGG GAT GTC TCC ATG TAC CTC CTT CTG AAG GAT GGT GGG  
I L K G D V S M Y L L L K D G G

CGT TTA CGG TGC CAA TTC GAC ACA GTT TAC AAA GCA AAG TCT GTG CCA  
R L R C Q F D T V Y K A K S V P

AGA AAG ATG CCG GAC TGG CAC TTC ATC CAG CAT AAG CTC ACC CGT GAA  
R K M P D W H F I Q H K L T R E

GAC CGC AGC GAT GCT AAG AAT CAG AAA TGG CAT CTG ACA GAA CAT GCT  
D R S D A K N Q K W H L T E H A

ATT GCA TCC GGA TCT GCA TTG CCC TGA AAGCTT  
I A S G S A L P \* HindIII (SEQ ID NO:15 & 16)

Figure 10

Non-aggregating mutant FP4-NA was generated from zFP538-M128V (humanized version). In comparison with zFP538-M128V, FP4-NA contains two additional amino acid substitutions - K5E and K9T. Also, two accidental nucleotide substitutions were introduced due to PCR mistakes (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

GGA TCC GCC CAC AGC GAG CAC GGC CTG ACC GAG GAG ATG ACC ATG AAG  
BamHI A H S E H G L T E E M T M K

TAC CAC ATG GAG GGC TGC GTG AAC GGC CAC AAG TTC GTG ATC ACC GGC  
Y H M E G C V N G H K F V I T G

GAG GGC ATC GGC TAC CCC TTC AAG GGC AAG CAG ACC ATC AAC CTG TGC  
E G I G Y P F K G K Q T I N L C

GTC ATC GAG GGC GGC CCC CTG CCC TTC AGC GAG GAC ATC CTG AGC GCC  
V I E G G P L P F S E D I L S A

GGC TTC AAG TAC GGC GAC CGG ATC TTC ACC GAG TAC CCC CAG GAC ATC  
G F K Y G D R I F T E Y P Q D I

GTC GAC TAC TTC AAG AAC AGC TGC CCC GCC GGC TAC ACC TGG GGC CGG  
V D Y F K N S C P A G Y T W G R

AGC TTC CTG TTC GAG GAC GGC GCC GTG TGC ATC TGT AAC GTG GAC ATC  
S F L F E D G A V C I C N V D I

ACC GTG AGC GTG AAG GAG AAC TGC ATC TAC CAC AAG AGC ATC TTC AAC  
T V S V K E N C I Y H K S I F N

GGC GTG AAC TTC CCC GCC GAC GGC CCC GTG ATG AAG AAG ATG ACC ACC  
G V N F P A D G P V M K K M T T

AAC TGG GAG GCC AGC TGC GAG AAG ATC ATG CCC GTG CCT AAG CAG GGC  
N W E A S C E K I M P V P K Q G

ATC CTG AAG GGC GAC GTG AGC ATG TAC CTG CTG CTG AAG GAC GGC GGC  
I L K G D V S M Y L L L K D G G

CGG TAC CGG TGC CAG TTC GAC ACC GTG TAC AAG GCC AAG AGC GTG CCC  
R Y R C Q F D T V Y K A K S V P

AGC AAG ATG CCC GAG TGG CAC TTC ATC CAG CAC AAG CTG CTG CGG GAG  
S K M P E W H F I Q H K L L R E

GAC CGG AGC GAC GCC AAG AAC CAG AAG TGG CAG CTG ACC GAG CAC GCC  
D R S D A K N Q K W Q L T E H A

ATC GCC TTC CCC AGC GCC CTG GCC TGA AAGCTT  
I A F P S A L A \* HindIII

(SEQ ID NOS: 17 & 18)

Figure 11

	M	A	L	S	N	E	F	I	G	D	D	M	K	M	
676	ATG	GCC	CTG	TCC	AAC	GAG	TTC	ATC	GGC	GAC	GAC	ATG	AAG	ATG	
	TAC	CGG	GAC	AGG	TTG	TTC	AAG	TAG	CCG	CTG	CTG	TAC	TTC	TAC	
	T	Y	H	M	D	G	C	V	N	G	H	Y	F	T	V
721	ACC	TAC	CAC	ATG	GAC	GGC	TGC	GTG	AAC	GGC	CAC	TAC	TTC	ACC	GTG
	TGG	ATG	GTG	TAC	CTG	CCG	ACG	CAC	TTG	CCG	GTG	ATG	AAG	TGG	CAC
	K	G	E	G	S	G	K	P	Y	E	G	T	Q	T	S
766	AAG	GGC	GAG	GGC	AGC	GGC	AAG	CCC	TAC	GAG	GGC	ACC	CAG	ACC	TCC
	TTC	CCG	CTC	CCG	TCG	CCG	TTC	GGG	ATG	CTC	CCG	TGG	GTC	TGG	AGG
	T	F	K	V	T	M	A	N	G	G	P	L	A	F	S
811	ACC	TTC	AAG	GTG	ACC	ATG	GCC	AAC	GGC	GGC	CCC	CTG	GCC	TTC	TCC
	TGG	AAG	TTC	CAC	TGG	TAC	CGG	TTG	CCG	CCG	GGG	GAC	CGG	AAG	AGG
	F	D	I	L	S	T	V	F	M	Y	G	N	R	C	F
856	TTC	GAC	ATC	CTG	TCC	ACC	GTG	TTC	ATG	TAC	GGC	AAC	CGC	TGC	TTC
	AAG	CTG	TAG	GAC	AGG	TGG	CAC	AAG	TAC	ATG	CCG	TTG	GCG	ACG	AAG
	T	A	Y	P	T	S	M	P	D	Y	F	K	Q	A	F
901	ACC	GCC	TAC	CCC	ACC	AGC	ATG	CCC	GAC	TAC	TTC	AAG	CAG	GCC	TTC
	TGG	CGG	ATG	GGG	TGG	TCG	TAC	GGG	CTG	ATG	AAG	TTC	GTC	CGG	AAG
	P	D	G	M	S	Y	E	R	T	F	T	Y	E	D	G
946	CCC	GAC	GGC	ATG	TCC	TAC	GAG	AGA	ACC	TTC	ACC	TAC	GAG	GAC	GGC
	GGG	CTG	CCG	TAC	AGG	ATG	CTC	TCT	TGG	AAG	TGG	ATG	CTC	CTG	CCG
	G	V	A	T	A	S	W	E	I	S	L	K	G	N	C
991	GGC	GTG	GCC	ACC	GCC	AGC	TGG	GAG	ATC	AGC	CTG	AAG	GGC	AAC	TGC
	CCG	CAC	CGG	TGG	CGG	TCG	ACC	CTC	TAG	TCG	GAC	TTC	CCG	TTG	ACG
	F	E	H	K	S	T	F	H	G	V	N	F	P	A	D
1036	TTC	GAG	CAC	AAG	TCC	ACC	TTC	CAC	GGC	GTG	AAC	TTC	CCC	GCC	GAC
	AAG	CTC	GTG	TTC	AGG	TGG	AAG	GTG	CCG	CAC	TTG	AAG	GGG	CGG	CTG
	G	P	V	M	A	K	K	T	T	G	W	D	P	S	F
1081	GGC	CCC	GTG	ATG	GCC	AAG	AAG	ACC	ACC	GGC	TGG	GAC	CCC	TCC	TTC
	CCG	GGG	CAC	TAC	CGG	TTC	TTC	TGG	TGG	CCG	ACC	CTG	GGG	AGG	AAG
	E	K	M	T	V	C	D	G	I	L	K	G	D	V	T
1126	GAG	AAG	ATG	ACC	GTG	TGC	GAC	GGC	ATC	TTG	AAG	GGC	GAC	GTG	ACC
	CTC	TTC	TAC	TGG	CAC	ACG	CTG	CCG	TAG	AAC	TTC	CCG	CTG	CAC	TGG
	A	F	L	M	L	Q	G	G	G	N	Y	R	C	Q	F
1171	GCC	TTC	CTG	ATG	CTG	CAG	GGC	GGC	GGC	AAC	TAC	AGA	TGC	CAG	TTC
	CGG	AAG	GAC	TAC	GAC	GTC	CCG	CCG	CCG	TTG	ATG	TCT	ACG	GTC	AAG
	H	T	S	Y	K	T	K	K	P	V	T	M	P	P	N
1216	CAC	ACC	TCC	TAC	AAG	ACC	AAG	AAG	CCC	GTG	ACC	ATG	CCC	CCC	AAC
	GTG	TGG	AGG	ATG	TTC	TGG	TTC	TTC	GGG	CAC	TGG	TAC	GGG	GGG	TTG
	H	V	V	E	H	R	I	A	R	T	D	L	D	K	G
1261	CAC	GTG	GTG	GAG	CAC	CGC	ATC	GCC	AGA	ACC	GAC	CTG	GAC	AAG	GGC
	GTG	CAC	CAC	CTC	GTG	GGC	TAG	CGG	TCT	TGG	CTG	GAC	CTG	TTC	CCG
	G	N	S	V	Q	L	T	E	H	A	V	A	H	I	T
1306	GGC	AAC	AGC	GTG	CAG	CTG	ACC	GAG	CAC	GCC	GTG	GCC	CAC	ATC	ACC
	CCG	TTG	TCG	CAC	GTC	GAC	TGG	CTC	GTG	CGG	CAC	CGG	GTG	TAG	TGG
	S	V	V	P	F	*									
1351	TCC	GTG	GTG	CCC	TTC	TGA									
	AGG	CAC	CAC	GGG	AAG	ACT									

(SEQ ID NO:19 & 20)

Figure 12

Non-aggregating mutant FP7-NA was generated from M35-5 (FP7a). In comparison with M35-5, FP7-NA contains two additional substitutions - K6T and K7E. Nucleotide substitutions in the codon for Leu-4 were introduced to optimize codon usage (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

GGA TCC GCC TCC CTG CTG ACC GAG ACC ATG CCC TTC AGG ACC ACC ATC  
BamHI A S L L T E T M P F R T T I

GAG GGC ACC GTG AAC GGC CAC TAC TTC AAG TGC ACC GGC AAG GGC GAG  
E G T V N G H Y F K C T G K G E

GGC AAC CCC CTC GAG GGC ACC CAG GAG ATG AAG ATC GAG GTG ATC GAG  
G N P L E G T Q E M K I E V I E

GGC GGC CCC CTG CCC TTC GCC TTC CAC ATC CTG TCC ACC TCC TGC ATG  
G G P L P F A F H I L S T S C M

TAC GGC TCC AAG GCC TTC ATC AAG TAC GTG TCC GGC ATC CCC GAC TAC  
Y G S K A F I K Y V S G I P D Y

TTC AAG CAG TCC CTC CCC GAG GGC TTC ACC TGG GAG CGC ACC ACC ACC  
F K Q S L P E G F T W E R T T T

TAC GAG GAC GGC GGC TTC CTG ACC GCC CAC CAG GAC ACC TCC CTG GAC  
Y E D G G F L T A H Q D T S L D

GGC GAC TGC CTG GTG TAC AAG GTG AAG ATC CTG GGC AAC AAC TTC CCC  
G D C L V Y K V K I L G N N F P

GCC GAC GGC CCC GTG ATG CAG AAC AAG GCC GGC CGC TGG GAG CCC TCC  
A D G P V M Q N K A G R W E P S

ACC GAG ATC GTG TAC GAG GTG GAC GGC GTG CTG CGC GGC CAG TCC CTG  
T E I V Y E V D G V L R G Q S L

ATG GCC CTG GAG TGC CCC GGC GGT CGC CAC CTG ACC TGC CAC CTG CAC  
M A L E C P G G R H L T C H L H

ACC ACC TAC CGC TCC AAG AAG CCC GCC TCC GCC CTG AAG ATG CCC GGC  
T T Y R S K K P A S A L K M P G

TTC CAC TTC GAG GAC CAC CGC ATC GAG ATC CTG GAG GAG GTG GAG AAG  
F H F E D H R I E I L E E V E K

GGC AAG TGC TAC AAG CAG TAC GAG GCC GCC GTG GGC CGC TAC TGC GAC  
G K C Y K Q Y E A A V G R Y C D

GCC GCC CCC TCC AAG CTG GGC CAC AAC TGAAGCTT  
A A P S K L G H N \* HindIII (SEQ ID NO:21 & 22)

Figure 13

Dimeric mutant AsRed M35-5D (in comparison to M355NA it carries one amino acid substitution L166S according to GFP numbering; L159S according to self-numbering)

ATG GCC TCC CTG CTG ACC GAG ACC ATG CCC TTC AGG ACC ACC ACC ATC  
M A S L L T E T M P F R T T T I

GAG GGC ACC GTG AAC GGC CAC TAC TTC AAG TGC ACC GGC AAG GGC GAG  
E G T V N G H Y F K C T G K G E

GGC AAC CCC CTC GAG GGC ACC CAG GAG ATG AAG ATC GAG GTG ATC GAG  
G N P L E G T Q E M K I E V I E

GGC GGC CCC CTG CCC TTC GCC TTC CAC ATC CTG TCC ACC TCC TGC ATG  
G G P L P F A F H I L S T S C M

TAC GGC TCC AAG GCC TTC ATC AAG TAC GTG TCC GGC ATC CCC GAC TAC  
Y G S K A F I K Y V S G I P D Y

TTC AAG CAG TCC CTC CCC GAG GGC TTC ACC TGG GAG CGC ACC ACC ACC  
F K Q S L P E G F T W E R T T T

TAC GAG GAC GGC GGC TTC CTG ACC GCC CAC CAG GAC ACC TCC CTG GAC  
Y E D G G F L T A H Q D T S L D

GGC GAC TGC CTG GTG TAC AAG GTG AAG ATC CTG GGC AAC AAC TTC CCC  
G D C L V Y K V K I L G N N F P

GCC GAC GGC CCC GTG ATG CAG AAC AAG GCC GGC CGC TGG GAG CCC TCC  
A D G P V M Q N K A G R W E P S

ACC GAG ATC GTG TAC GAG GTG GAC GGC GTG CTG CGC GGC CAG TCC AGC  
T E I V Y E V D G V L R G Q S S

ATG GCC CTG GAG TGC CCC GGC GGT CGC CAC CTG ACC TGC CAC CTG CAC  
M A L E C P G G R H L T C H L H

ACC ACC TAC CGC TCC AAG AAG CCC GCC TCC GCC CTG AAG ATG CCC GGC  
T T Y R S K K P A S A L K M P G

TTC CAC TTC GAG GAC CAC CGC ATC GAG ATC CTG GAG GAG GTG GAG AAG  
F H F E D H R I E I L E E V E K

GGC AAG TGC TAC AAG CAG TAC GAG GCC GCC GTG GGC CGC TAC TGC GAC  
G K C Y K Q Y E A A V G R Y C D

GCC GCC CCC TCC AAG CTG GGC CAC AAC TGA  
A A P S K L G H N \*

(SEQ ID NO:23 & 24)

Figure 14

Diagram showing a sequence of amino acids with an arrow indicating the reading frame. To the right are the names of the fluorescent proteins:

MRSSKNVIKEFMRFKVRMEGTVNGHE	drFP583
MSCSKNVIKEFMRFQVRMEGTVNGHE	ds/drFP616
MAQSKHGLTKEMTMKYRMEGCVGDGHK	zFP506
MAHSKHGLKEEMTMKYHMEGCVNGHK	zFP538
MALSNKFIGDDMKMTYHMDGCVNGHY	amFP486
MASFLKKT_TMPFKTTIEGTVNGHY	asFP595

(SEQ ID NOS:25-30)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Figure 15

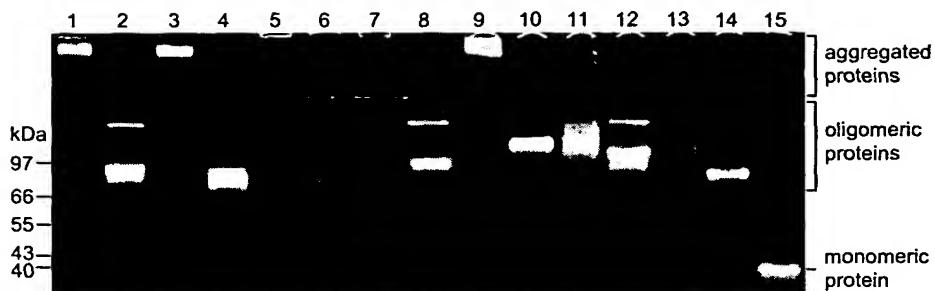


Figure 16

